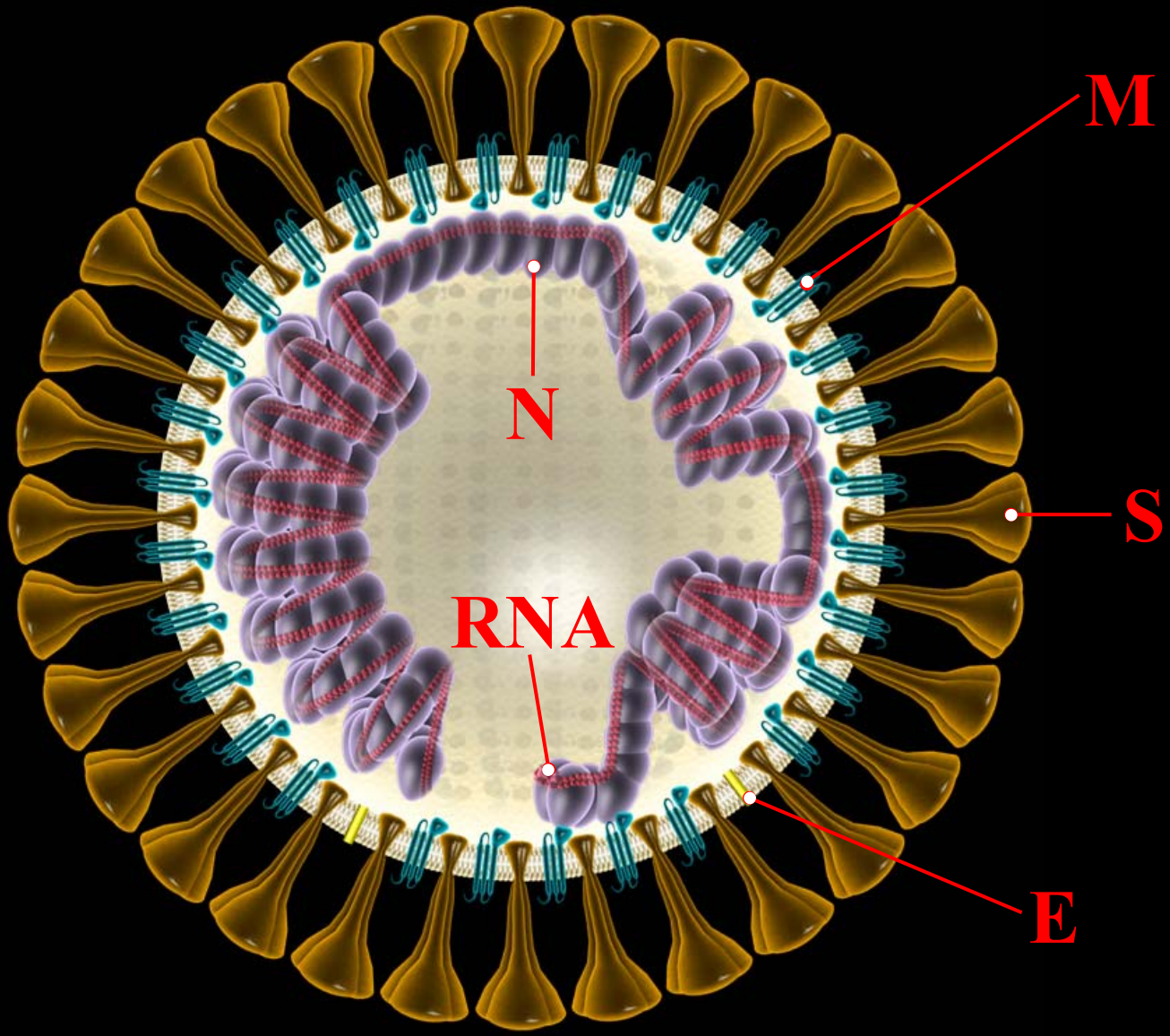
SARS Virus



Coronavirus Biology and Pathogenesis

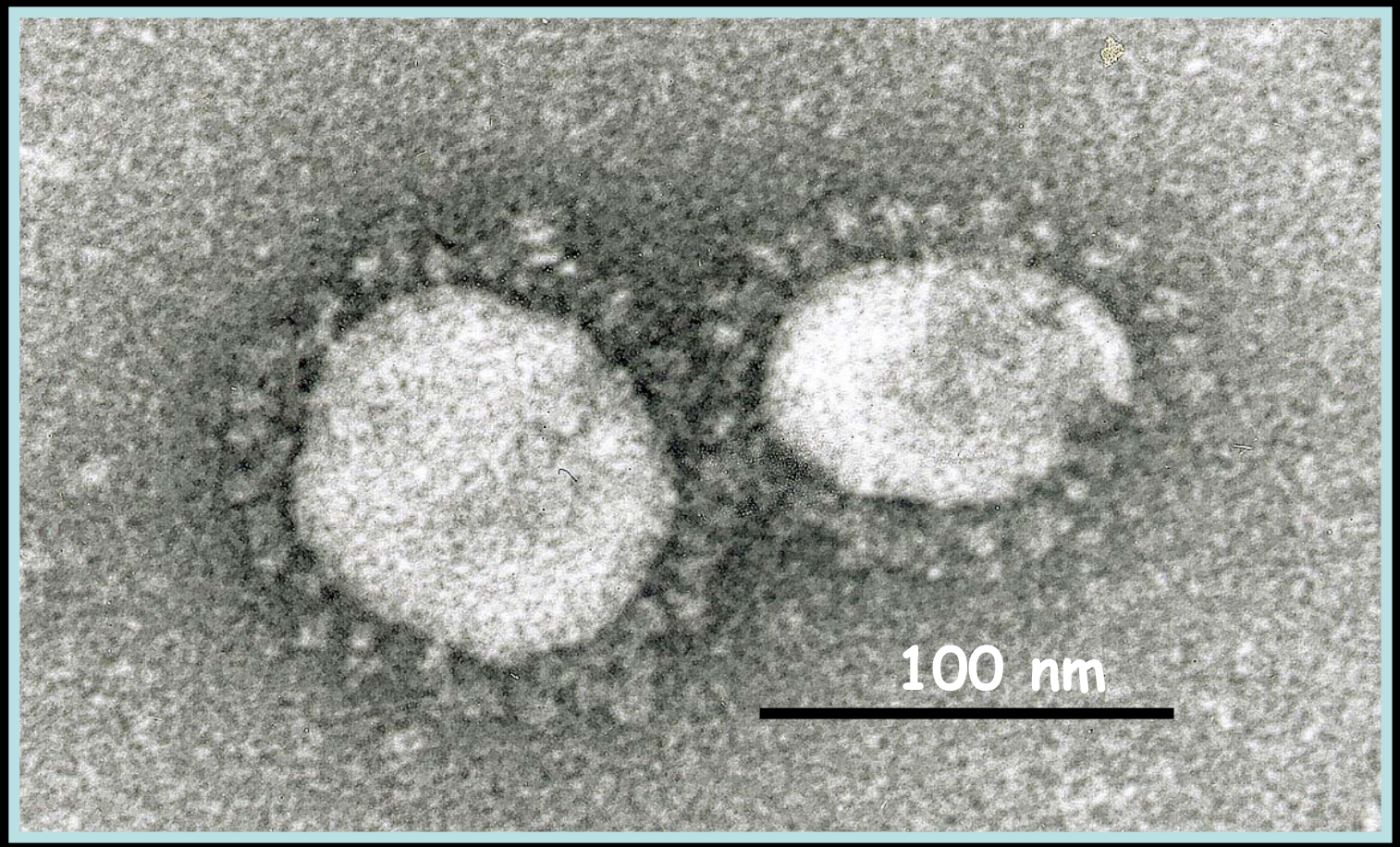
May 30, 2003

Kathryn Holmes
Department of Microbiology
University of Colorado Health Sciences Center, Denver

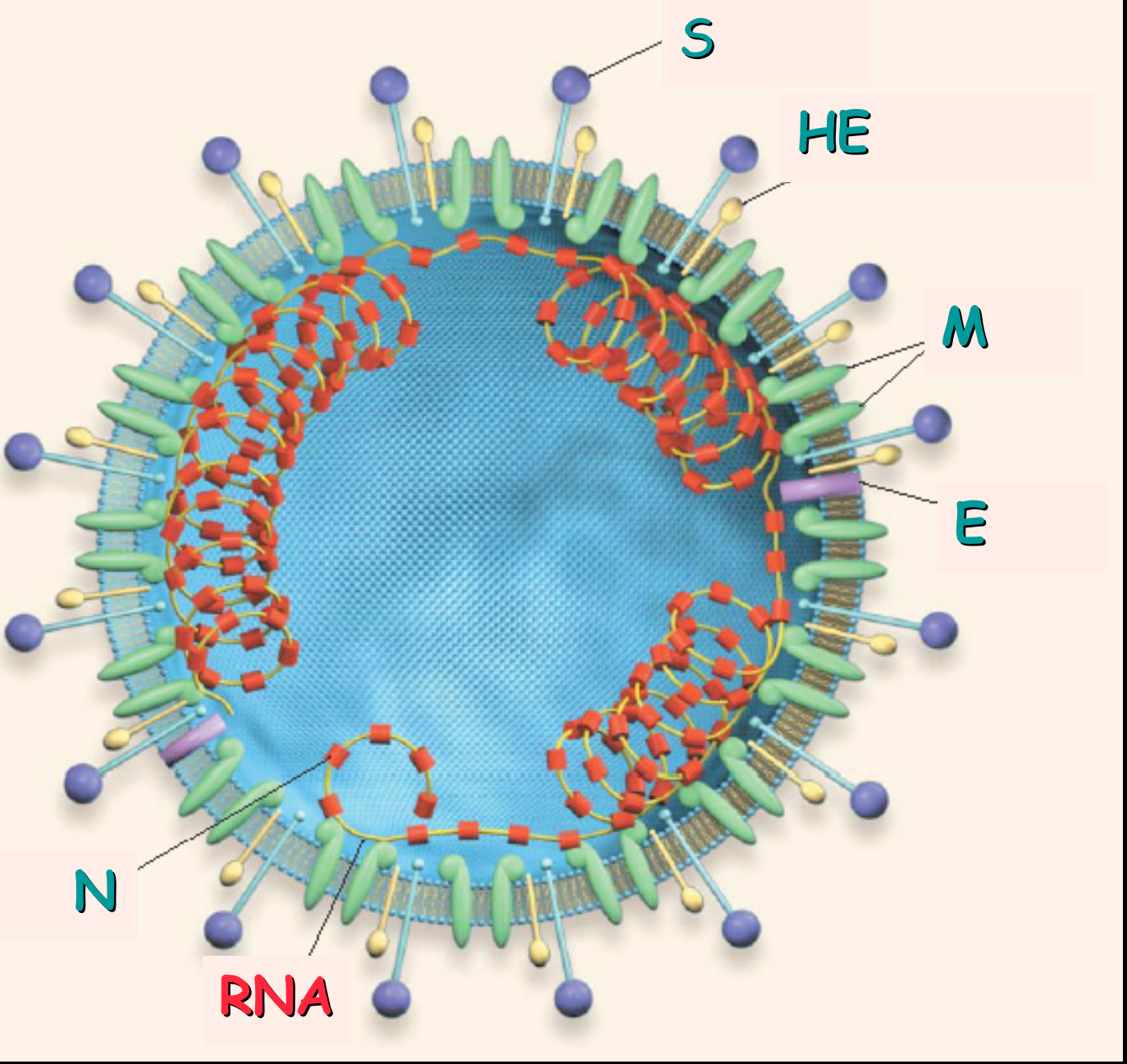
- Coronaviruses
  - Classification
  - Structural proteins
  - Replication
  - Drug targets
- Coronavirus diseases of man and animals
  - Pathogenesis
  - Immune responses
  - Epidemiology
  - Vaccines

Coronaviruses: Classification by EM

Almeida and Tyrrell (1967)

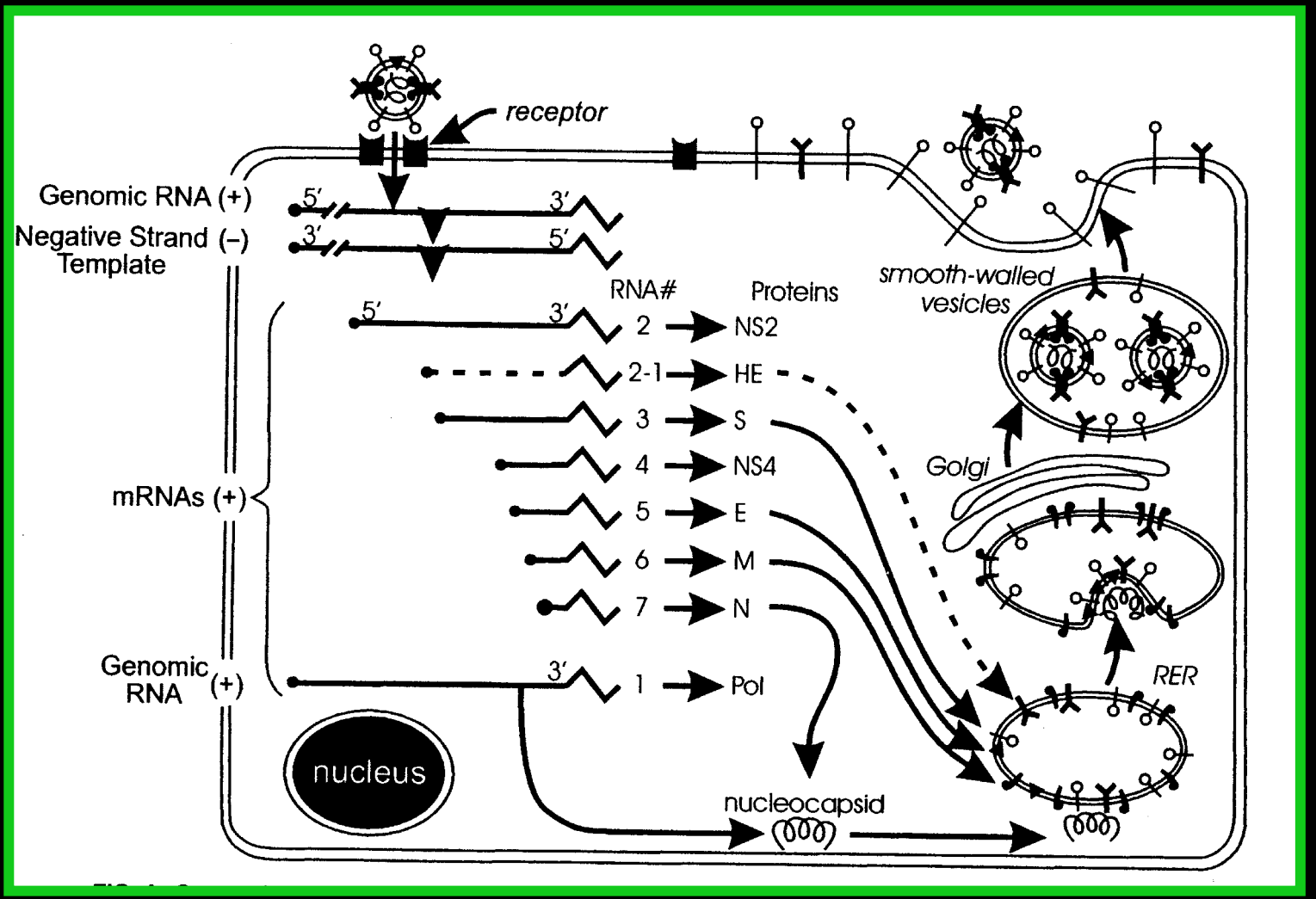


CORONAVIRUS



New Engl. J. Med.

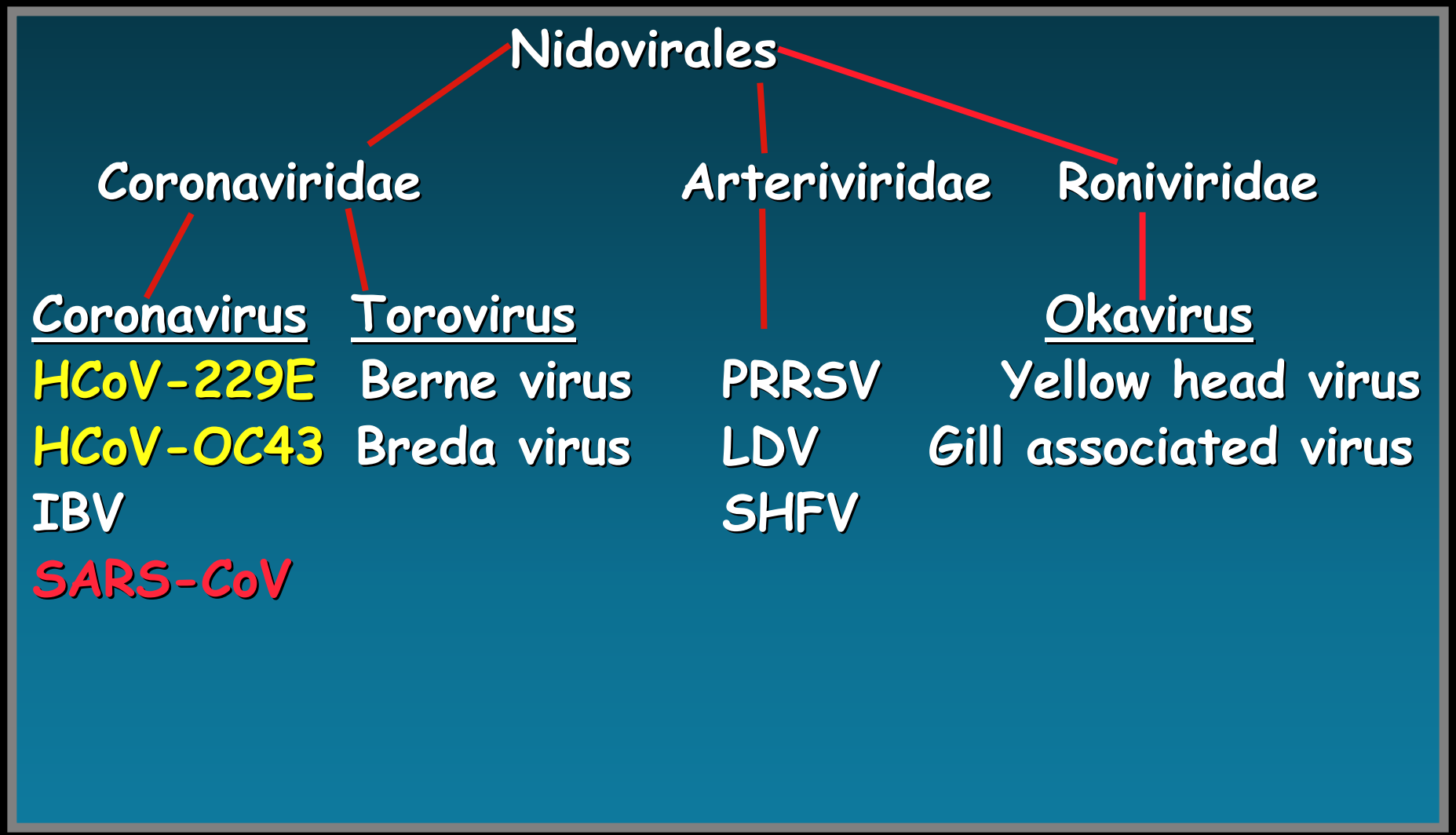
Coronavirus Replication



Coronaviruses, host ranges and diseases

Genetic Group	Virus	Host	Diseases (infection sites) Respiratory	Enteric	Other
I	HCoV-229E	human	X		
	TGEV	pig	(X)	X	
	PRCoV	pig	X		
	PEDV	pig		X	
	FIPV	cat	X	X	Systemic
	FCoV	cat		X	
	CCoV	dog		X	
2	HCoV-OC43	human	X	??	
	MHV	mouse	X	X	CNS, systemic
	RCoV	rat	X		Eye, GU
	HEV	pig		X	CNS
	BCoV**	cattle	X	X	
3	IBV	chicken	X	X	Kidney
	TCoV	turkey		X	
4??	SARS-CoV	human	X	(X)	(Kidney)

NEW TAXONOMY



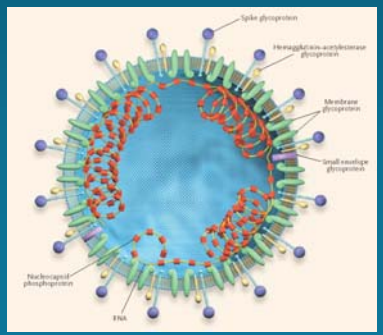
Nidoviruses share replication strategy, but differ in genome size and virion structure

Nidovirales

Coronaviridae — Coronavirus, Torovirus

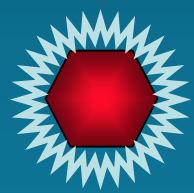
Arteriviridae

Roniviridae — Okavirus

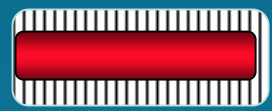


30 kb

20kb

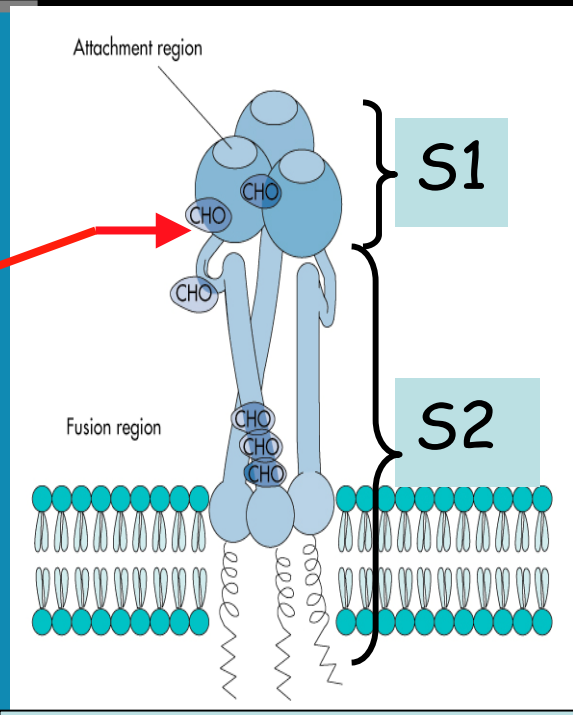


13 kb



The Spike Glycoprotein is a Coronavirus Virulence Factor

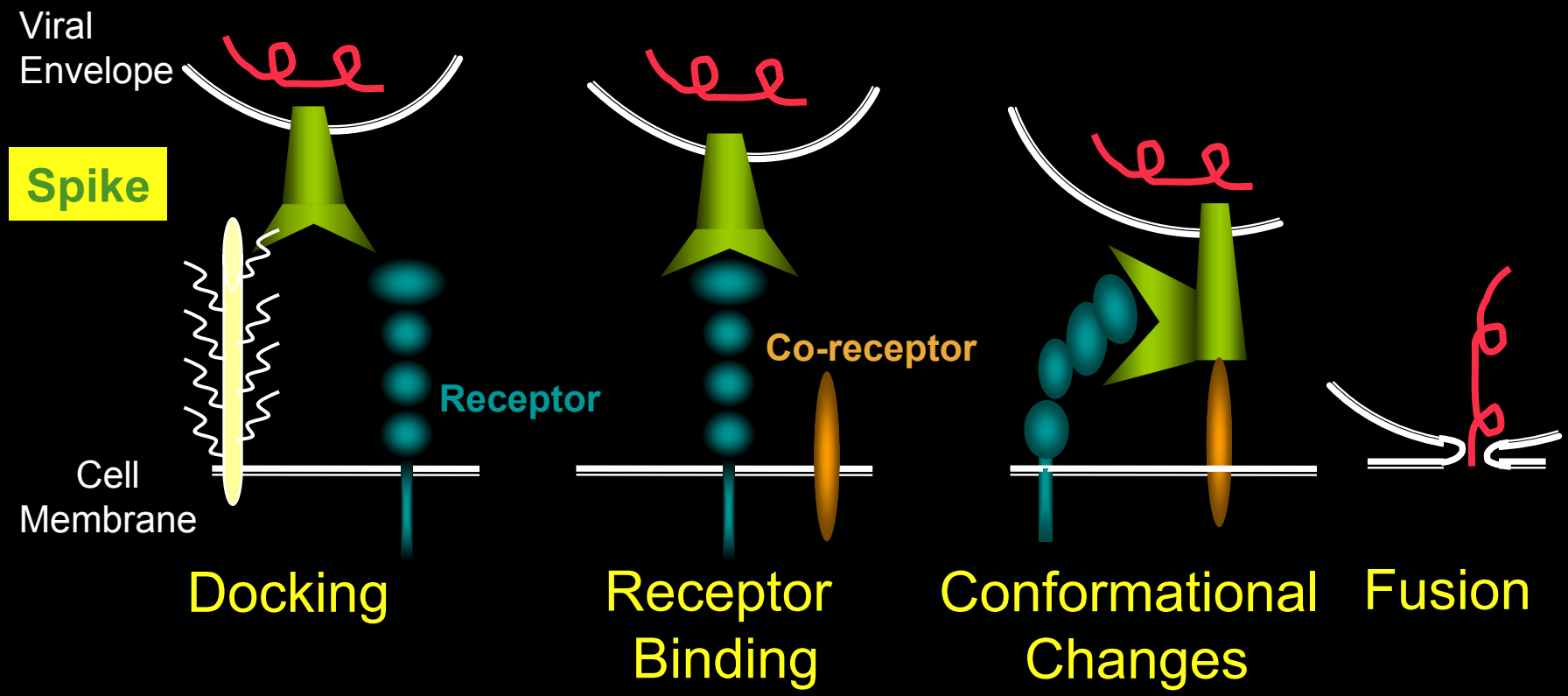
- **Spike glycoprotein (S) determines:**
  - Receptor binding and specificity
  - Membrane fusion, cell fusion
  - Protease susceptibility and activation
  - Deletions in S1 alter tissue tropism
  - pH of conformational changes in S
  - Virus neutralization by antibodies



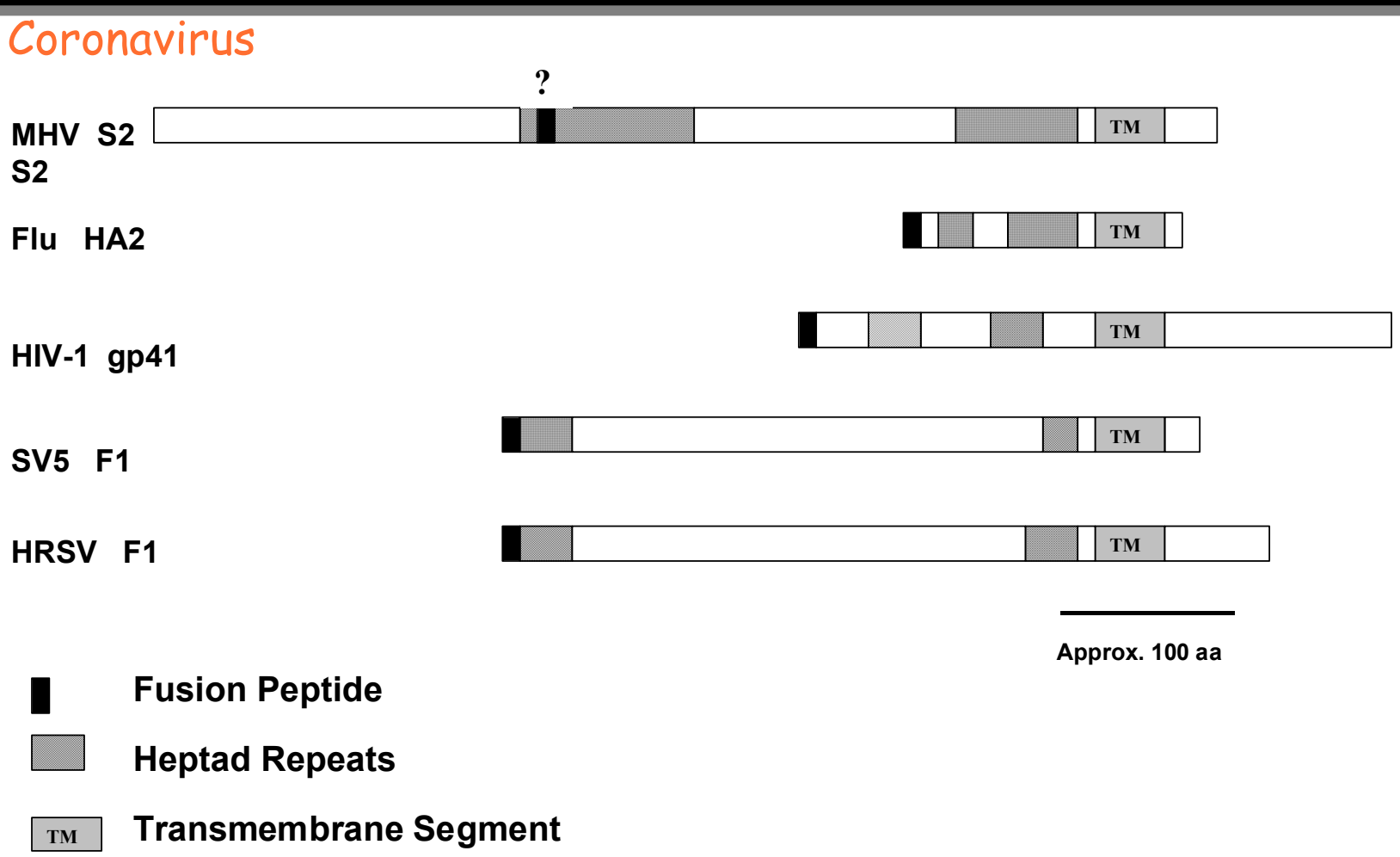
Type 1 viral fusion protein

Enveloped Virus Entry

Type I Fusion Protein



Fusion domains of Class I viral fusion proteins

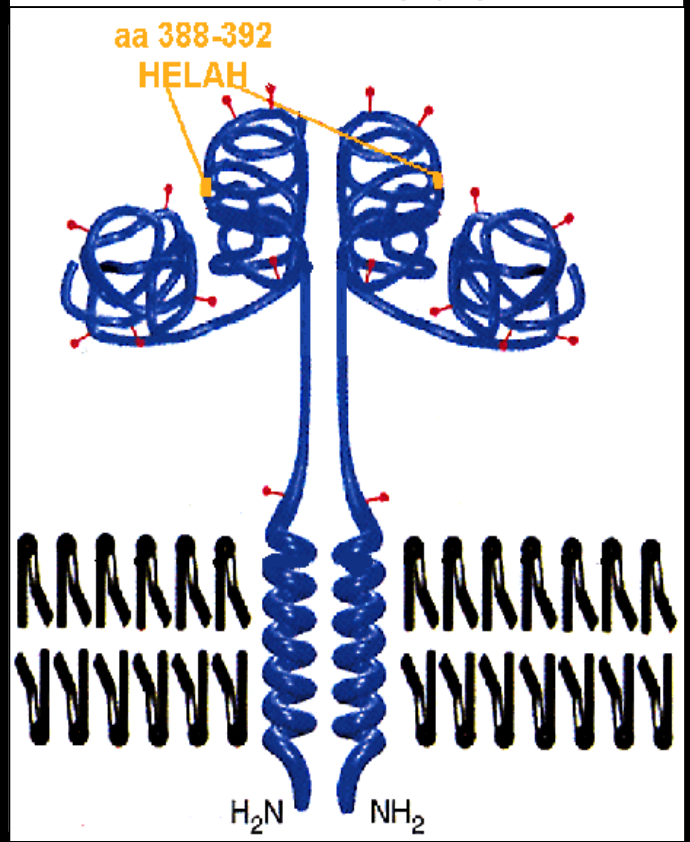


Coronaviruses, host ranges and receptors

Genetic Group	Virus	Host	Receptor	Co-receptor
I	HCoV-229E	human	human aminopeptidase N (hAPN)	
	TGEV	pig	porcine APN	
	PRCoV	pig	porcine APN	
	PEDV	pig		
	FIPV	cat	feline APN	
	FCoV	cat	feline APN	
	CCoV	dog	canine APN	
2	HCoV-OC43	human		9-0AcNA
	MHV	mouse	murine CEACAM1	
	RCoV	rat		9-0AcNA
	HEV	pig		9-0AcNA
	BCoV	cattle		9-0AcNA
3	IBV	chicken		
	TCoV	turkey		
4??	SARS-CoV	human		

Aminopeptidase N (APN or CD13)

APN Model

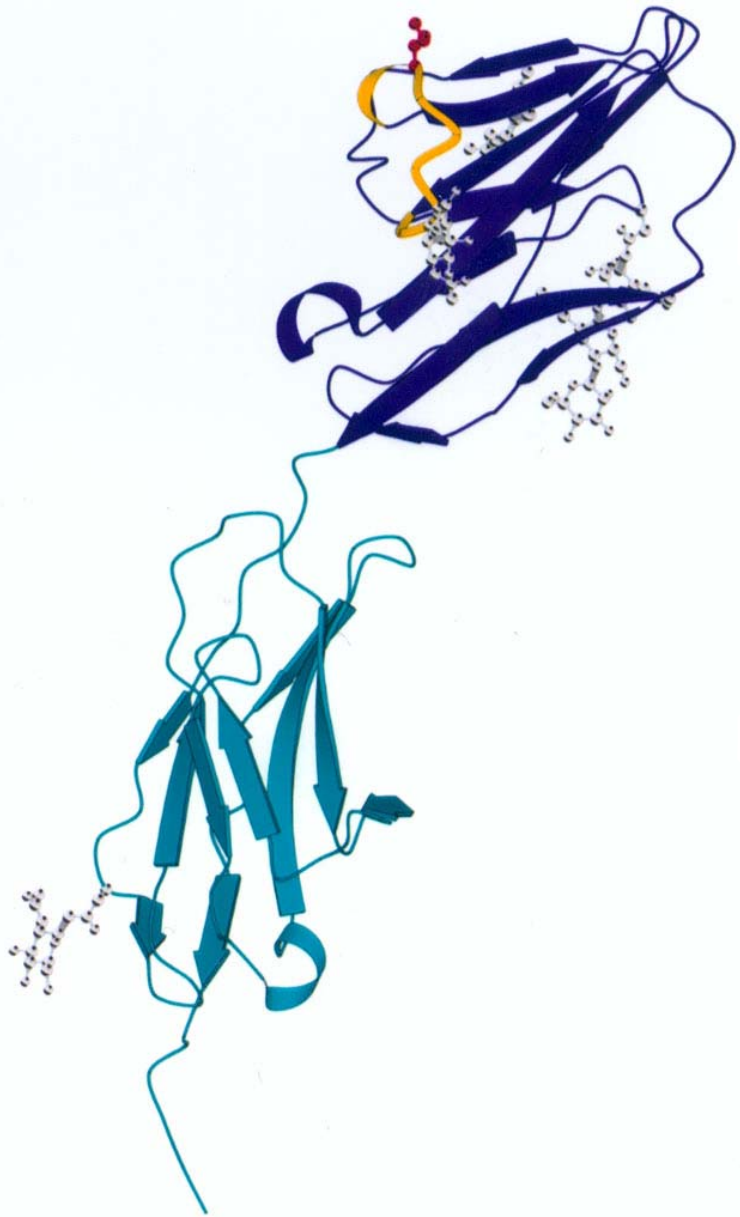


Modified from Riemann et al., Immunology Today 20: 83-88 (1999)

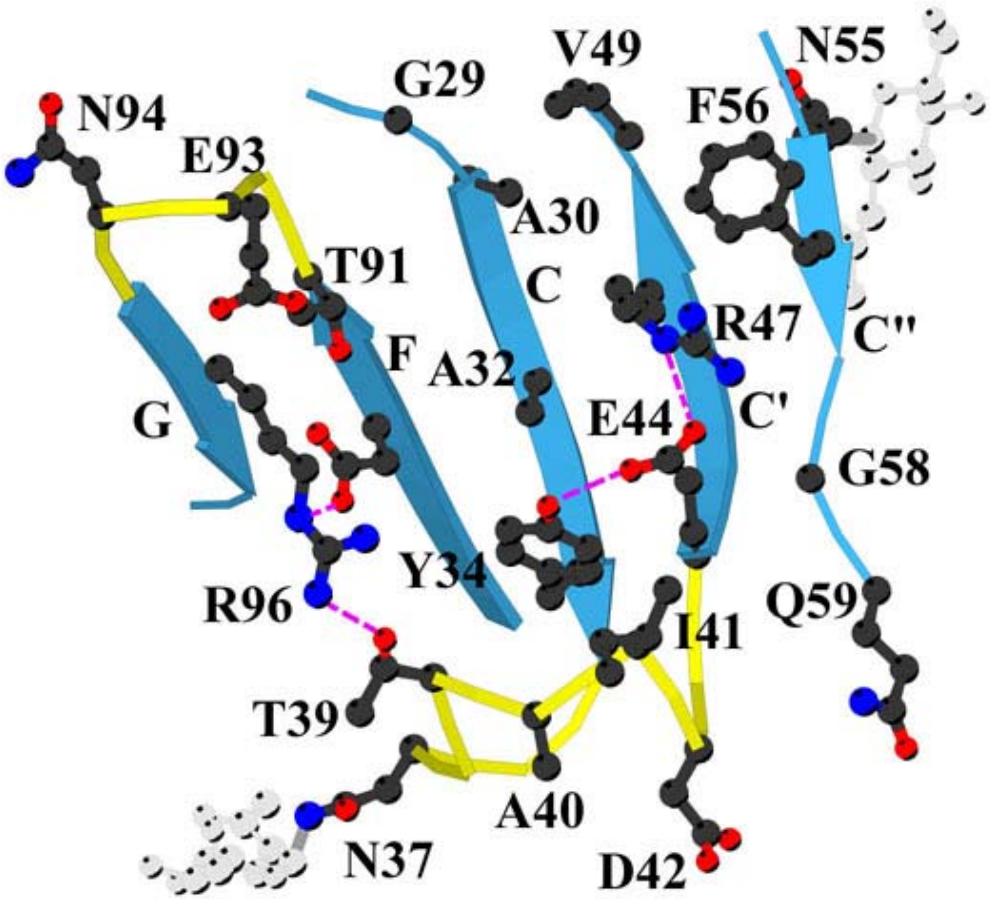
- 150 kDa type II glycoprotein
- Metalloprotease expressed in a variety of tissue and cell types
- Converts oligopeptides to amino acids
- Degrades regulatory peptides and neuroactive peptides
- Antigen processing and presentation
- Plays a role in the migration of human tumor cells
- Group 1 coronavirus receptor

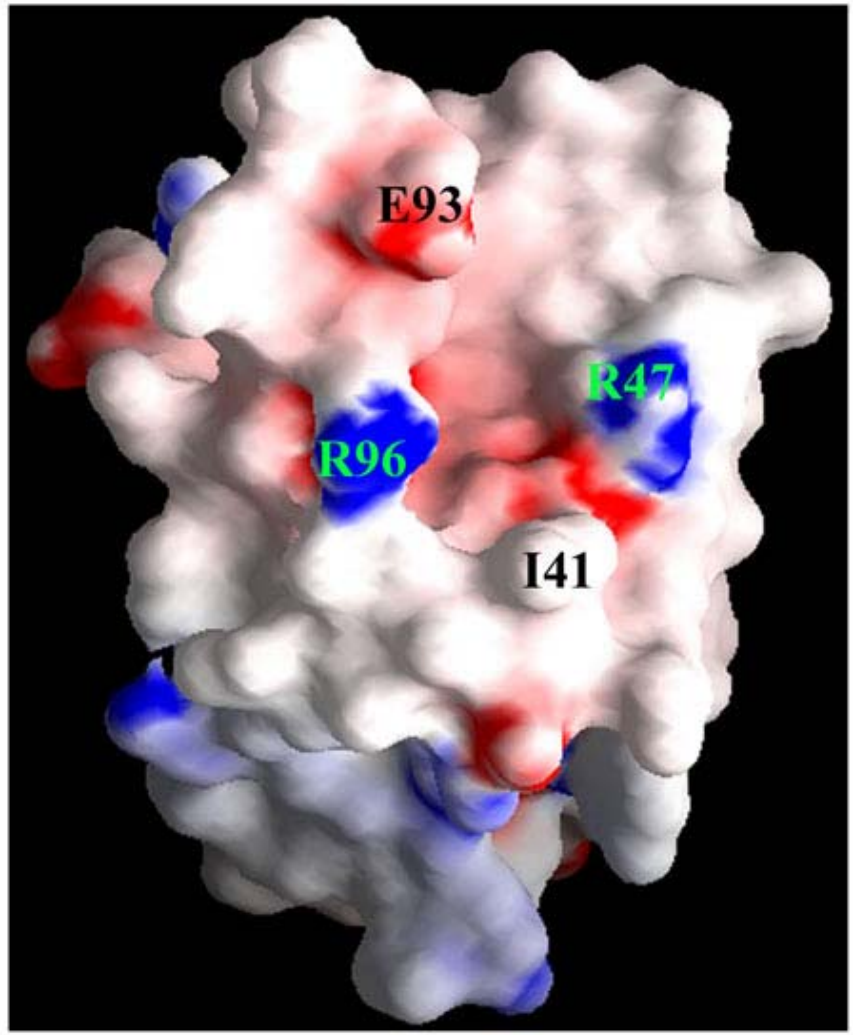
Murine CEACAM1a

The receptor for murine coronavirus MHV



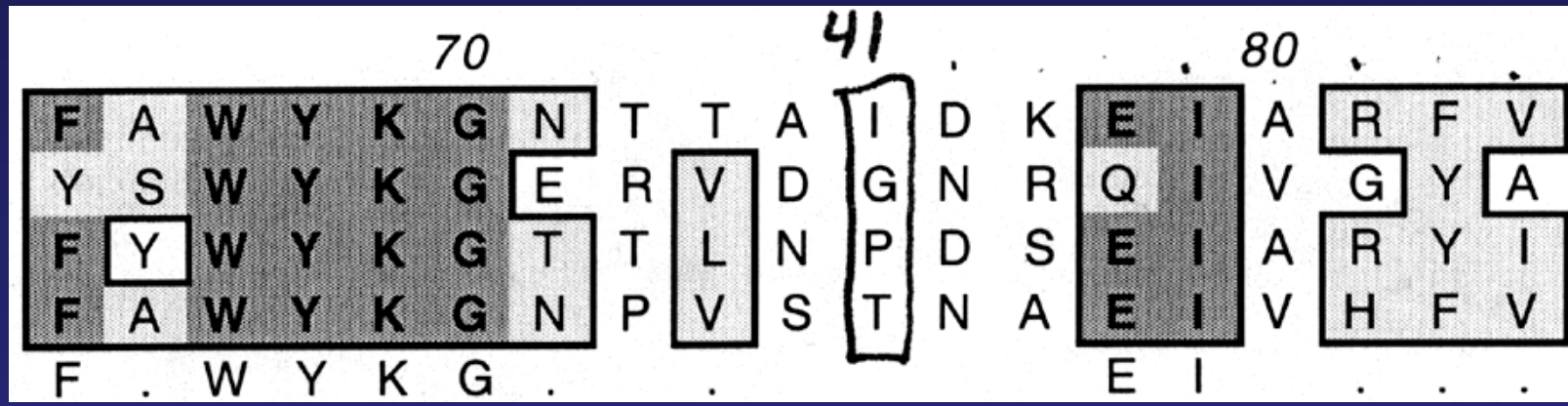
What the virus sees, or the view from the top



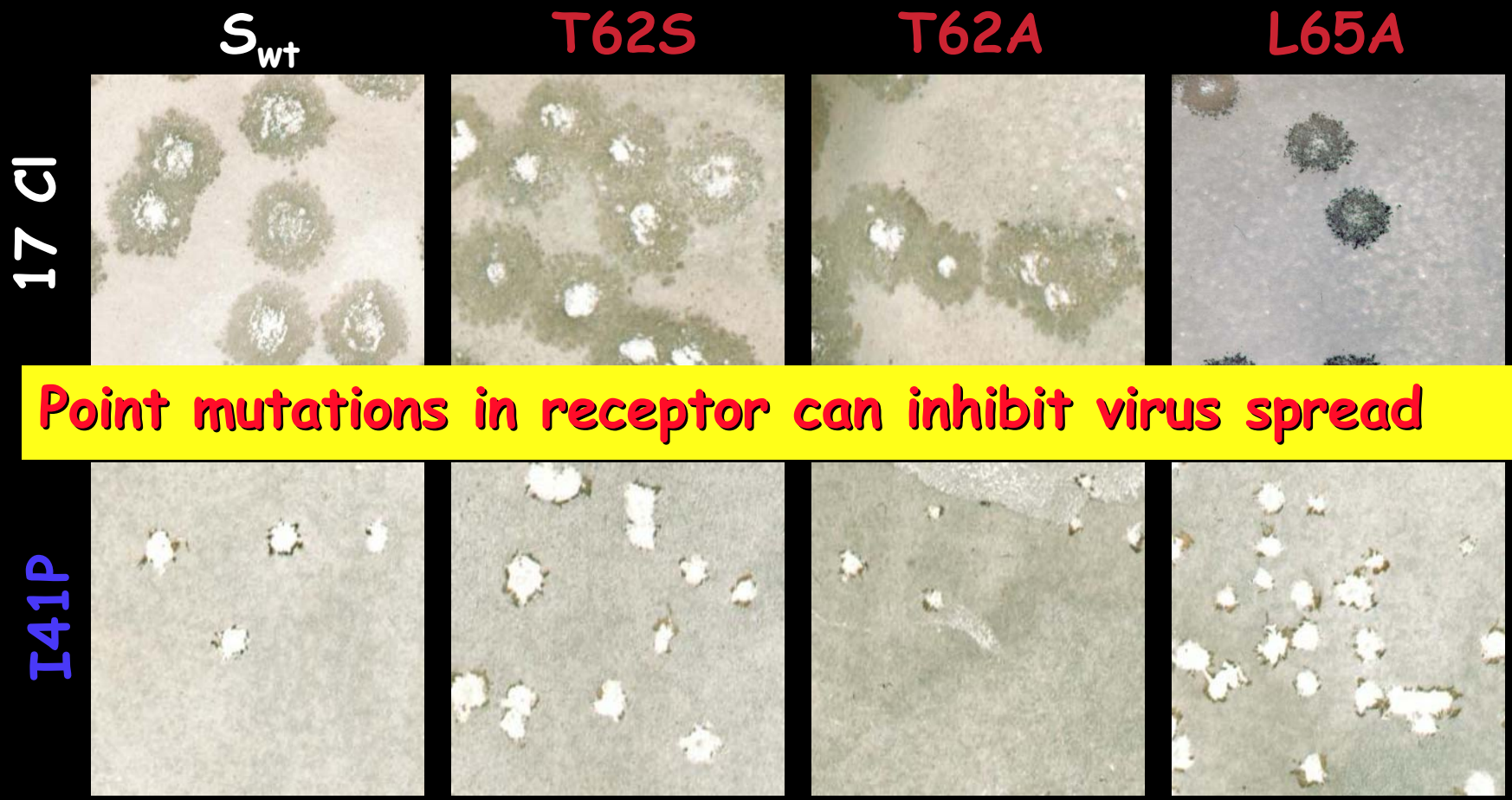


(Tan, Zelus *et al.*,2002)

C-C’ loop, I41 mutations



Plaques of mutant viruses on BHK cells expressing mutant receptor

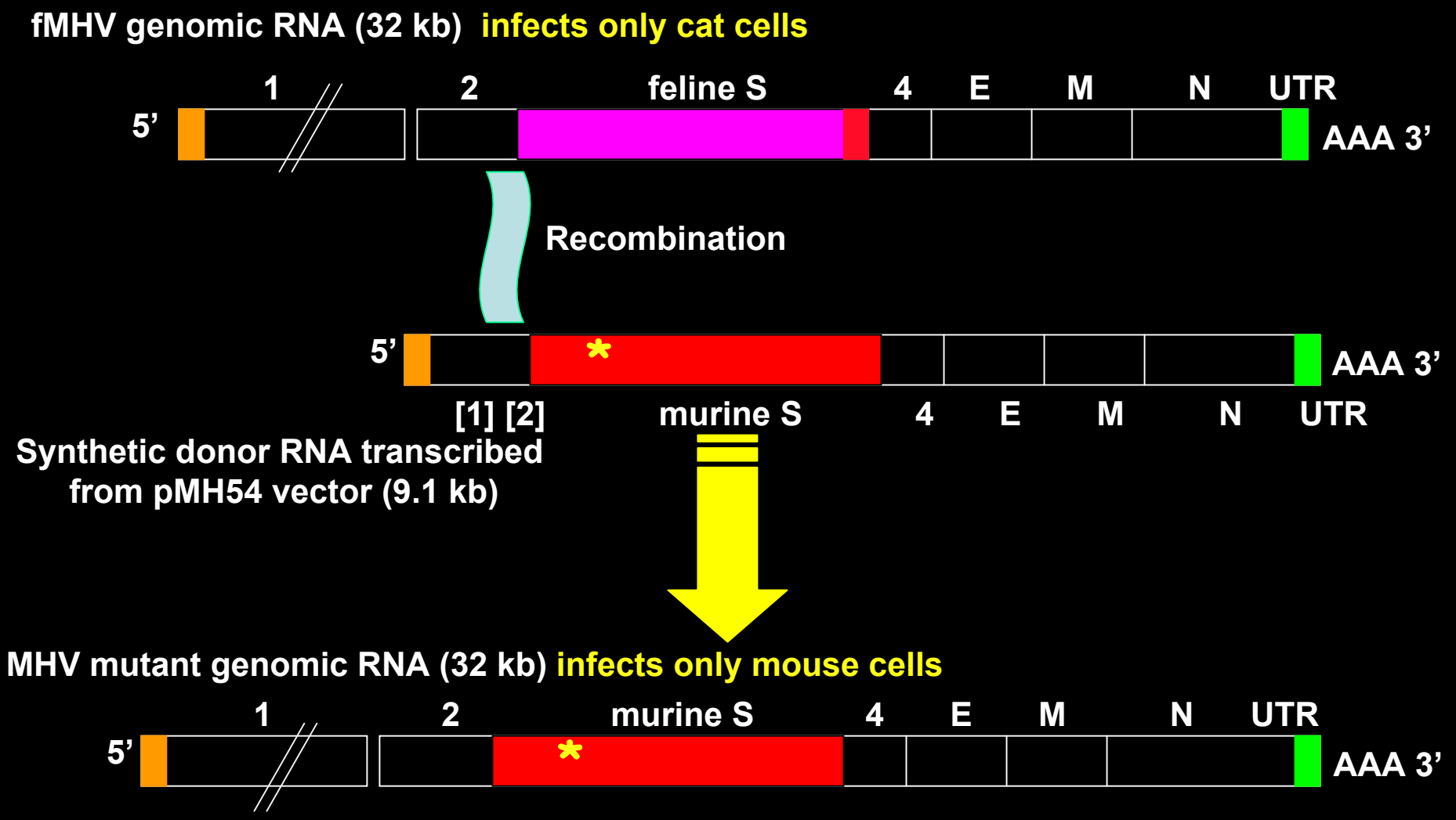


Introduction of mutations into the genomic RNA of coronaviruses

Reverse genetics using full length infectious clone

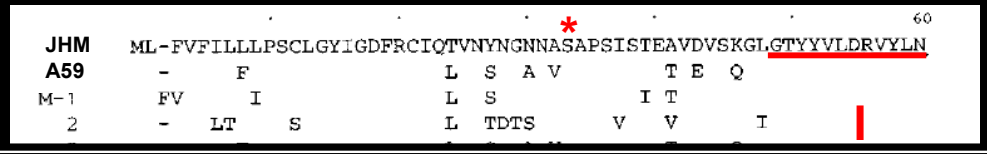
Targeted RNA recombination

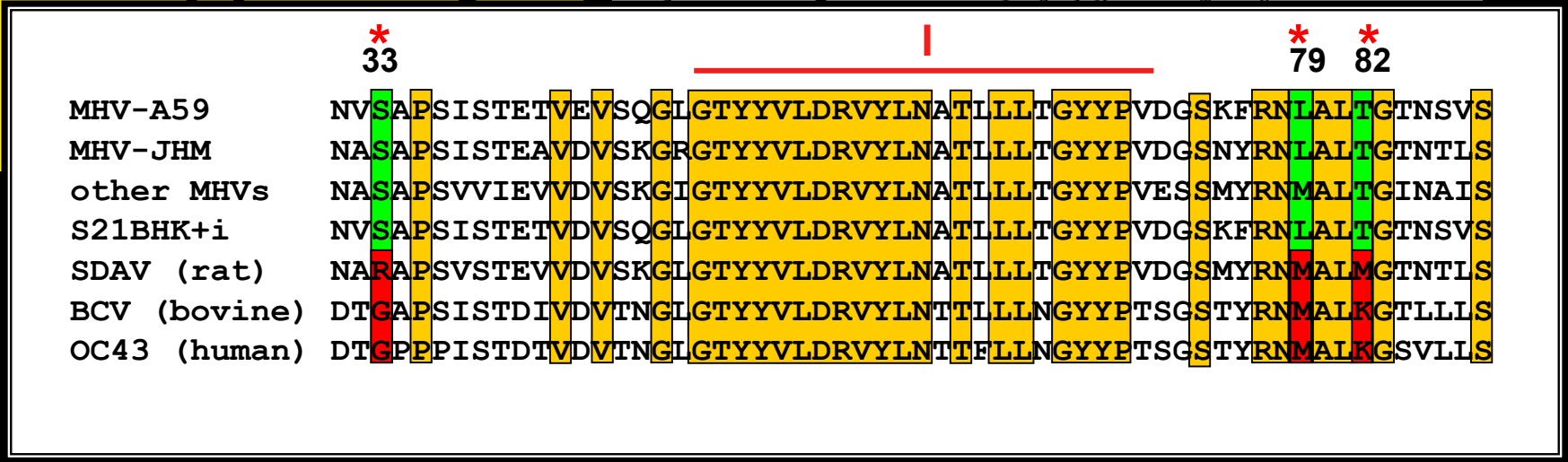
TARGETED RNA RECOMBINATION

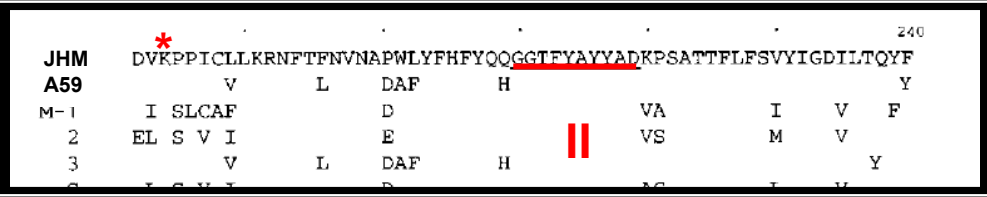


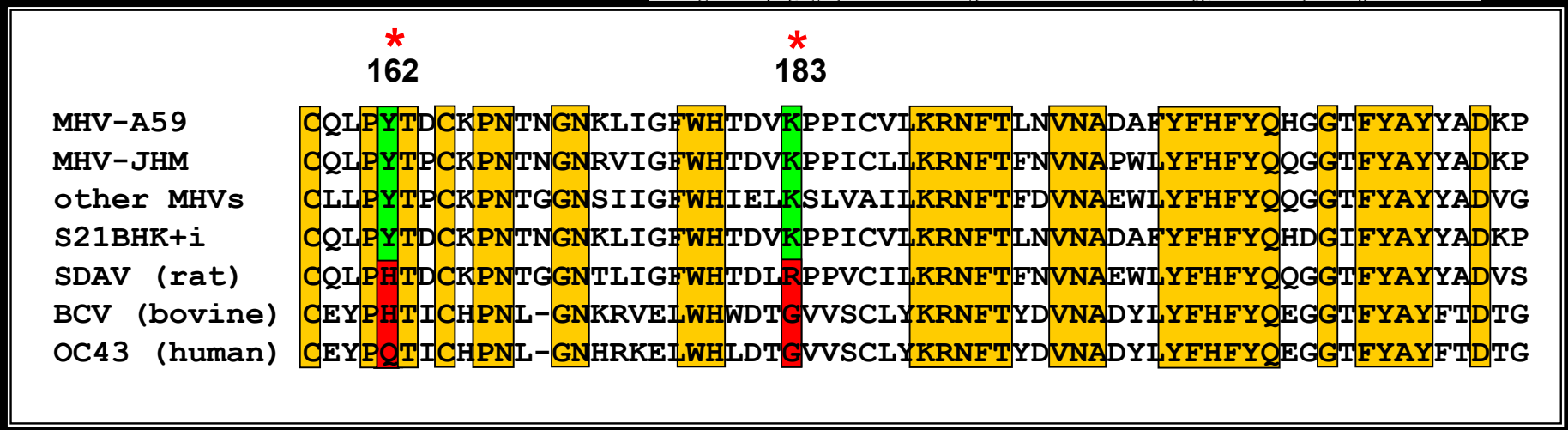
Kuo et al. J. Virol. 74:1393 (2000)

Mouse specific





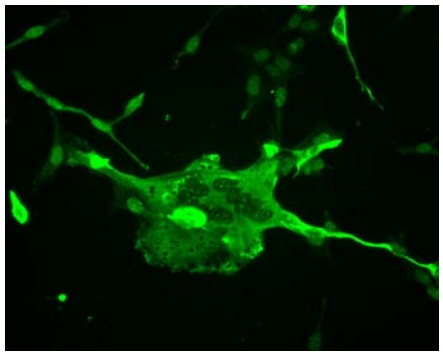




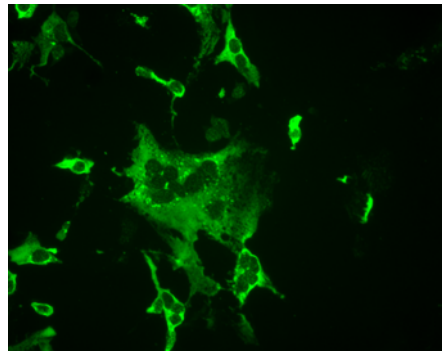
adapted from H. Suzuki and F. Taguchi, J Virol. 1996. 70(4).

MHV viruses with substitutions in S differ in growth on mouse cells

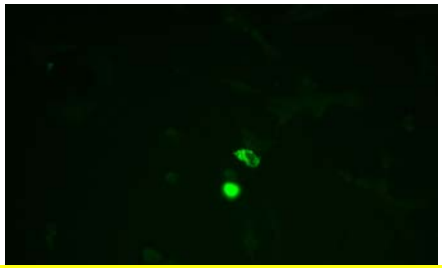
S33R



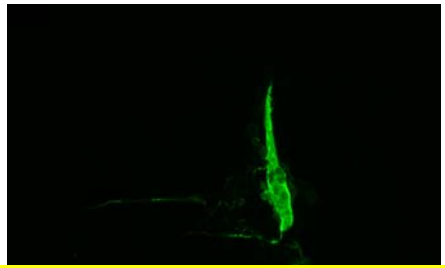
S_{A59}



L79M/T82K

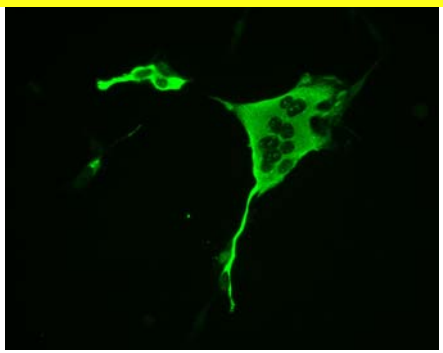


L79A/T82A

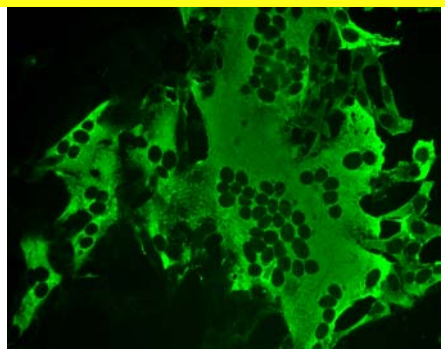


Point mutations in S can change receptor interactions

K183R



K183G



Coronaviruses

- Classification
- Structural proteins
- Replication
- Drug targets

Coronavirus diseases of man and animals

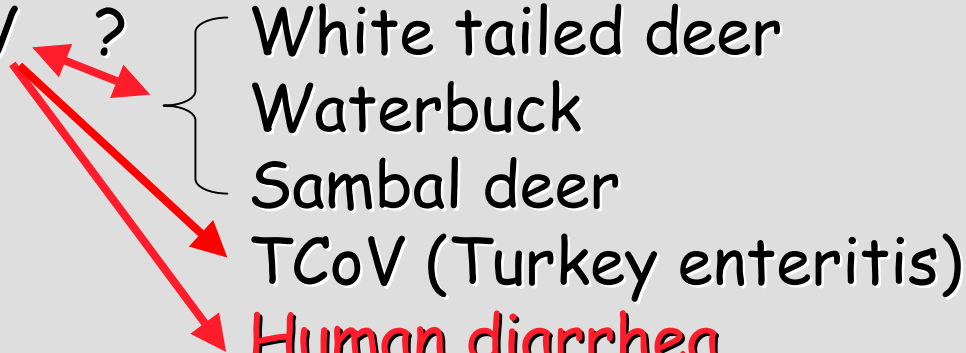
- Pathogenesis
- Immune responses
- Epidemiology
- Vaccines

A coronavirus with several diseases and a naturally extended host range

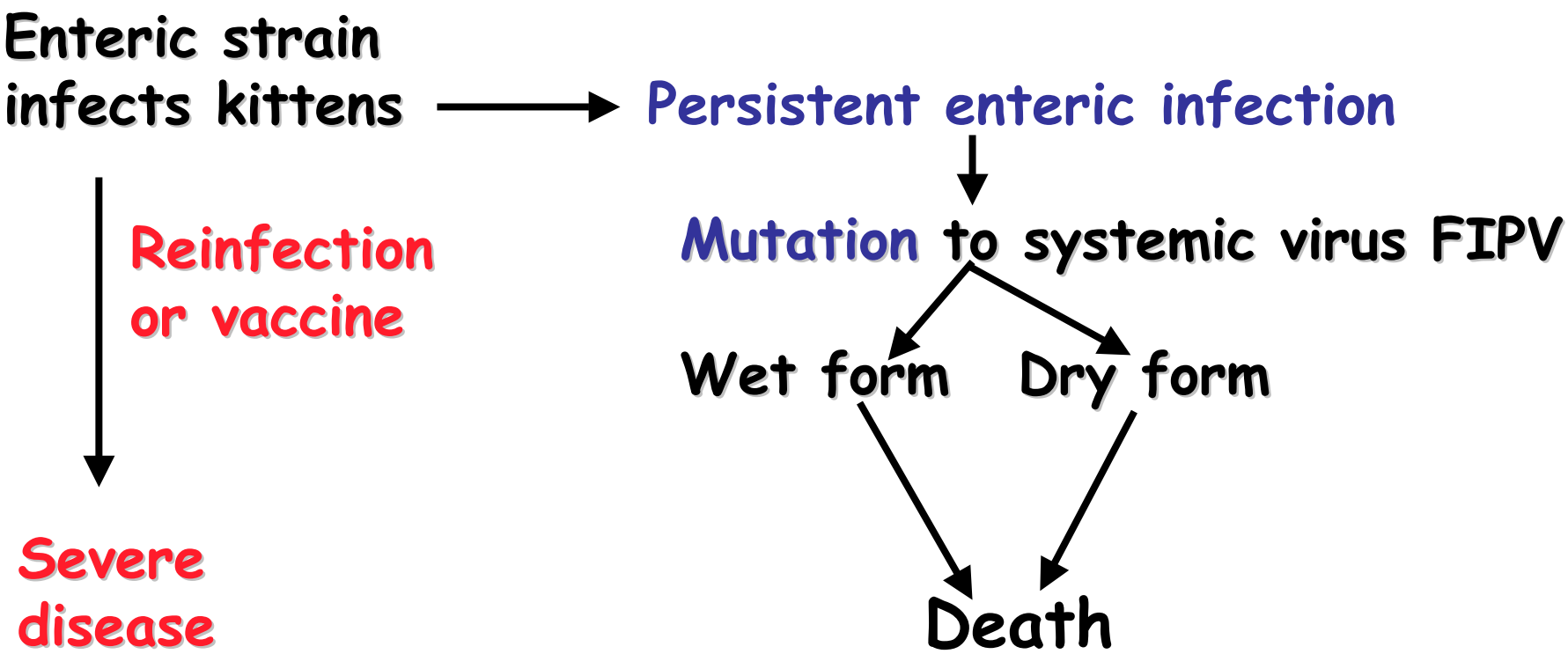
Bovine coronavirus
(Pneumoenteric)

Calf diarrhea
Winter dysentery
Calf respiratory disease
Shipping fever

One serotype, 2 subtypes



Feline enteric coronavirus and feline infections peritonitis



Deletions or mutations in Spike protein change virulence and tissue tropism

TGEV

Enteric disease in piglets

Deletion of 225 aa in spike

or mutation

PRCoV

Mild respiratory disease of pigs Interstitial pneumonia

PRCoV provides partial protection from TGEV

HCoV-229E in Volunteers

Chilvers et al, Eur. Resp. J. 18:965-970 (2001)

11 volunteers were inoculated intranasally with HCoV-229E.
Cold symptoms were recorded daily and nasal epithelium studied.

Overall symptoms	
No symptoms	3
"Possible cold"	4
Definite cold	4

Number experiencing	
Headache	6
Cough	4
Fever	2
Disruption of nasal epithelium	11

No animal model or reservoir

Inapparent infection common

Seasonal outbreaks

Genetically stable viruses

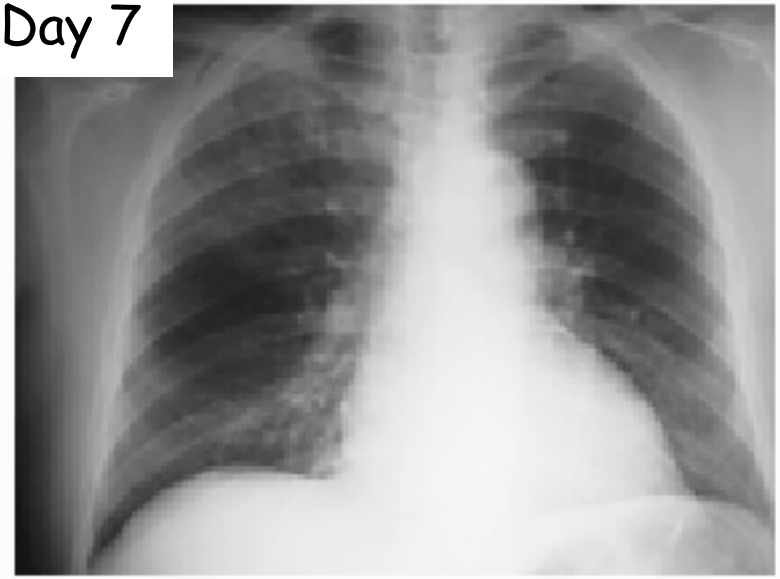
Repeated infections common

No drugs or vaccines

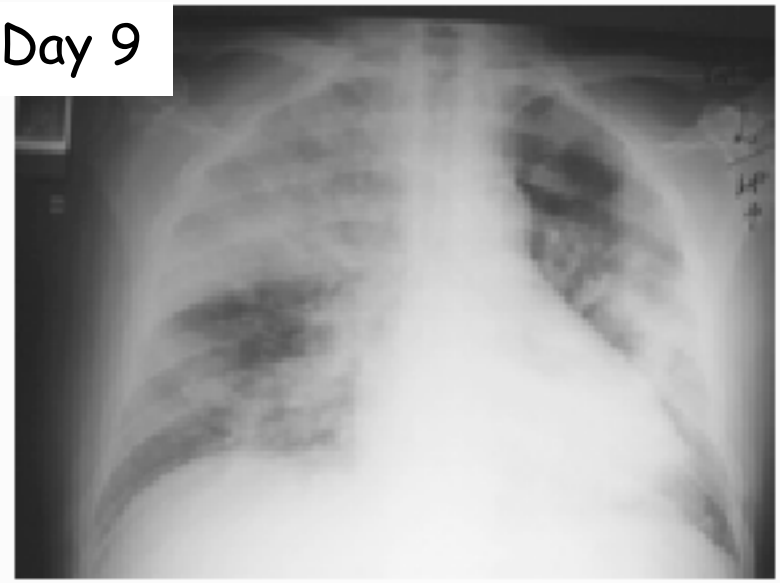
SARS

Poutanen, *et al.*, NEJMed, April, 2003

Day 7



Day 9



Day 14

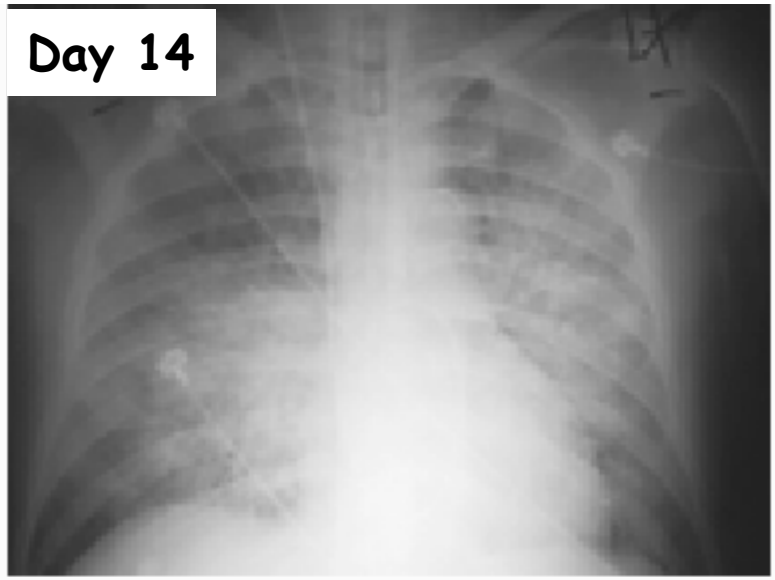


Figure 3. The Course of Disease in Patient 8.

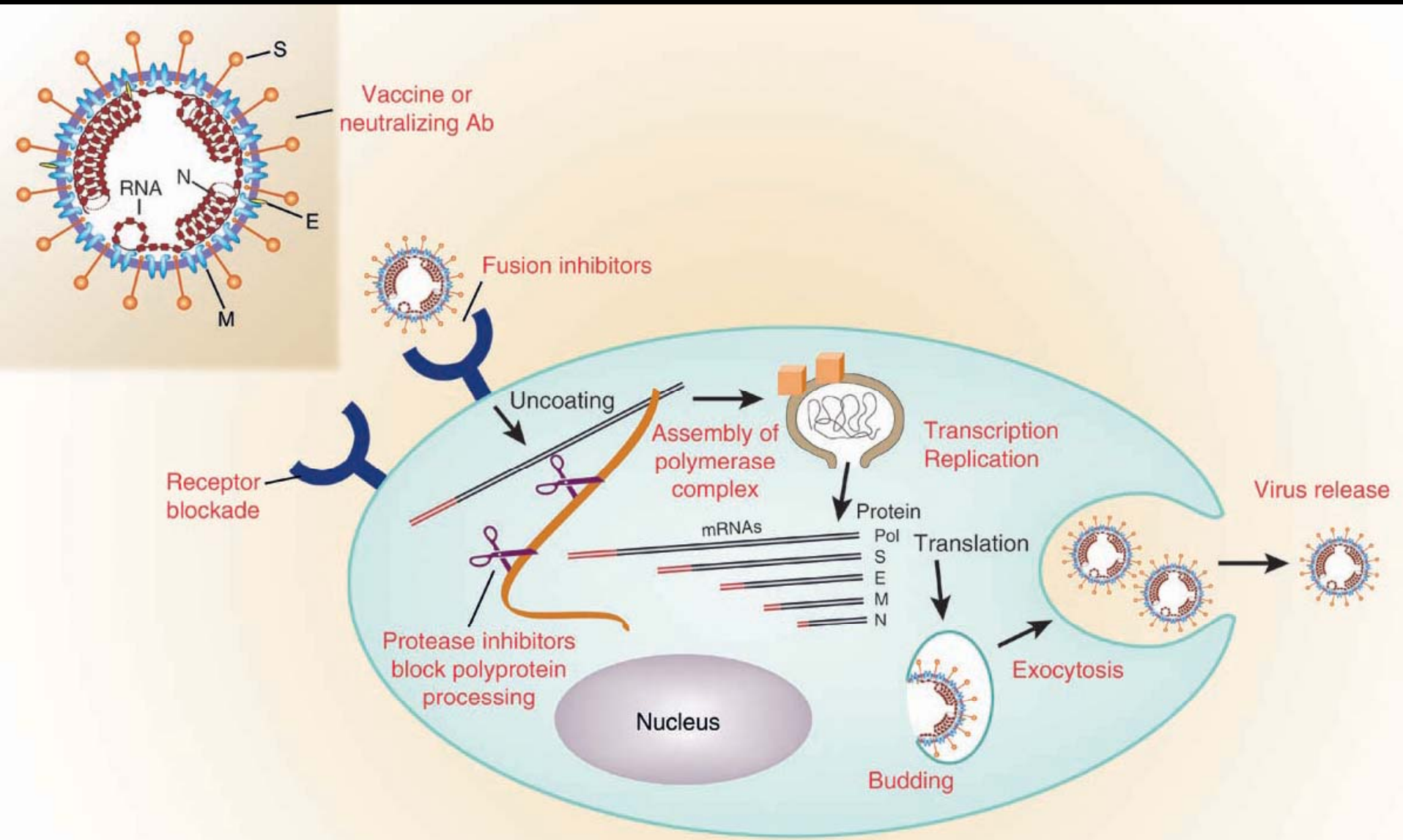
A 76-year-old man (Patient 8), who was exposed to Patient 2 on March 7, had fever (temperatures of up to 40°C), diaphoresis, and fatigue three days later on March 10. A chest radiograph was obtained on March 14, revealing right-upper-lobe and bibasilar interstitial infiltrates (Panel A). He subsequently noted a nonproductive cough and increasing dyspnea and was admitted to the hospital on March 16, demonstrating bilateral patchy air-space disease with relative sparing of the right lung base and left upper lobe (Panel B). He was admitted to the intensive care unit and was intubated and received mechanical ventilation because of respiratory distress. Progressive respiratory failure and worsening of the findings on chest radiography occurred (Panel C), and the patient died on March 21.

SARS

Syndrome	Acute interstitial pneumonia
Incubation period	~4-10 days
Case fatality rate	~4-50%
Transmission	Human to human
	Close contact, super spreaders
Immune response	Neutralizing antibody; CTL?

Virus shedding	Duration uncertain
	Respiratory, fecal, urine
Pathogenesis	Poorly understood
Persistent infection	??
Susceptible to reinfection	??

Targets for SARS Vaccines and Drugs



K.V. Holmes, SARS Coronavirus: A new challenge for prevention and therapy J. Clin. Invest. 111:1605 (2003)

Some Targets for Drugs Against Coronaviruses

Blockade or inhibition of:

* Receptor interactions

Anti-R Mab blocks MHV infection in vitro and in vivo

* SARS-CoV cell fusion inhibited by heptad repeat peptide of viral spike protein

Hilgenfeld models SARS 3CL protease, suggests inhibitor

Inhibitor of papain like protease tested

* Replication and transcription

Oligonucleotide inhibitor of replication tested

* Inflammation

Acetyl esterase inhibitors inhibit gp2 CoV's in vitro

Coronavirus vaccines

Live attenuated vaccines

PEDV: passaged 90X in Vero cells

IBV: multiple serotypes,recombination are problems

Killed vaccines

Canine CoV vaccine

IBV

Vectored vaccines

Adenovector for lactogenic immunity to TGEV spike

Baculovirus for TGEV S or S,M,N partly protects vs enteric challenge

Passive immunization

Neutralizing monoclonal antibody

Antibody dependent enhancement (ADE) with FCoV vaccines.

Research Priorities

How many coronaviruses are there?
- Genetic relationships?
- Host ranges?
- Virulence determinants?

Pathogenesis

Immune responses

Small animal model for SARS

Effects of mutations

Diagnostic tests

Vaccines

Drugs